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(54) Title: AMPLIFICATION OF FOLATE-MEDIATED TARGETING TO TUMOR CELLS USING NANOPARTICLES

(57) Abstract

The invention relates to the delivery of drug, peptide and protein pharmaceuticals using the folate-mediated uptake system. More particularly the invention relates to the amplification of drug/pharmaceutical delivery with the folate uptake system using a folate-nanoparticle complex. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same, methods of treatment involving the complexes and uses of the complexes in the manufacture of medicaments.

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AMPLIFICATION OF FOLATE-MEDIATED TARGETING TO TUMOR CELLS USING NANOPARTICLES

Technical Field

The invention relates to the delivery of drug, peptide and protein pharmaceuticals using the folate-mediated uptake system. More particularly the invention relates to the amplification of drug/pharmaceutical delivery with the folate uptake system using a folate-nanoparticle complex. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same, methods of treatment involving the complexes and uses of the complexes in the manufacture of medicaments.

Background Art

In conventional cancer chemotherapy, in order to obtain a linear increase in killing of cancer cells it is often necessary to increase the amount of cytotoxic drugs present in the system in an exponential fashion. This in turn leads to an undesirable increase in non-specific cytotoxicity of bistander, healthy cells. Hence it is often necessary to repeatedly deliver a smaller dose of cytotoxin, which inevitably leads to the survival of a small fraction of drug-resistant cells. In an attempt to increase the dose of cytotoxic agent delivered to the tumor cell, specific targeting agents such as monoclonal antibodies to "tumor-specific antigens" have been employed. In many cases the resultant antibody-drug conjugate may be highly immunogenic, and thus lead to an antibody response against the conjugate thereby precluding further use. For this reason small, poorly immunogenic, tumor-specific molecules have been sought as alternatives to antibody molecules. Recently focus has switched to the use of molecules essential for growth to be used as targeting agents. The use of one of these, folic acid, is the subject of the current application.

Folic acid enters cells either through a carrier protein, termed the reduced folate carrier, or via receptor-mediated endocytosis facilitated by the folate receptor. The folate receptor is significantly over-expressed on a large fraction of human cancer cells including ovarian, breast, lung, endometrial, renal, colon, and cancers of myeloid hematopoietic cells. There are two folate receptors FR-α, and FR-β. In general FR-α, is upregulated in malignant tissues of epithelial origin such as ovarian carcinoma, while FR-β is overexpressed in malignant tissues of nonepithelial origin. While the FR have been detected in normal tissues involved in the retention and uptake of the vitamin, these tissues are in protected sites and generally not accessible following blood-borne delivery of folate conjugates. Thus there is expression in the choroid plexus, the intestinal brush border apical membrane surface and the proximal tubules of the kidney. In the latter case the receptor probably functions to scavenge excreted folate, and as such would not be accessible to large molecule weight folate complexes. Folate-mediated tumor targeting has been exploited to date for delivery of the following molecules and molecular complexes (i) protein toxins, (ii) low-molecular-weight chemotherapeutic agents, (iii) radio-imaging agents (iv) MRI contrast agents, (v) radiotherapeutic agents, (vi) liposomes with entrapped drugs, (vii) genes, (viii) antisense oligonucleotides, (ix) ribozymes, and (x) immunotherapeutic agents.

Two major limitations to the use of folate to target to tumor cells is that the dose deliverable is small, *i.e.* one molecule of drug for each molecule of folate, and that the majority of the folate-drug complexes are very small and as such are excreted in the kidneys and re-absorbed in the proximal tubules, thus leading to undesirable accumulation of folate-drug complexes in the kidney.

It is an object of the present invention to overcome or at least alleviate one or more of the above-mentioned disadvantages of the prior art.

Summary of the Invention

Surprisingly it has been found by the present inventors that both of the above-mentioned limitations could be addressed by incorporation of the active substance to be delivered within a nanoparticle, which is coated with folic acid or an analogue thereof. Thus,

amplification of drug/pharmaceutical delivery can occur by incorporation of quantities of the active substance within the folate-coated nanoparticle. Accumulation of the folate-drug complexes in the kidneys is also minimised due to the large size of the nanoparticle.

According to a first aspect of the present invention there is provided a complex comprising a nanoparticle, to which is coupled a targeting molecule, said nanoparticle enclosing an active substance, and wherein said targeting molecule (hereinafter termed TM) is folic acid, or an analogue thereof possessing binding activity to the folic acid receptor.

According to a second aspect of the present invention there is provided a process for the production of a nanoparticle complex of the invention, which process comprises one or more of the following steps :

(a) reacting nanoparticles with a TM to form the complex;

(b) chemically modifying a TM to provide at least one functional group capable of forming a chemical linkage and reacting nanoparticles and the modified TM to form the complex;

(c) reacting nanoparticles with at least one cross-linking agent to prepare "activated" nanoparticles which are reacted with a TM to form the complex;

(d) reacting a TM with at least one cross-linking agent and reacting the nanoparticles with the reacted TM to form the complex;

(e) reacting nanoparticles and a TM with at least one cross-linking agent to form the complex;

(f) reacting nanoparticles with at least one cross-linking agent, reacting a TM with at least one cross-linking agent and reacting the reacted nanoparticles and the reacted TM to form the complex; or

(g) reacting a TM with at least one cross-linking agent to prepare an analogue which is reacted with a hydrophobic moiety to form a hydrophobic derivative of the TM; and then incubating the hydrophobic derivative of the TM with a nanoparticle in such a manner that the nanoparticle is coated hydrophobically with the TM.

According to a third aspect of the present invention there is provided a medicament which comprises a nanoparticle complex of the invention together with a pharmaceutically acceptable carrier, excipient, diluent and/or adjuvant.

According to a fourth aspect of the present invention there is provided a method for the treatment, prophylaxis or amelioration of disease, in particular cancer, in a vertebrate host which method comprises the administration to said host of a therapeutically effective amount of a nanoparticle complex according to the invention.

According to a fifth aspect the present invention provides a method for the treatment, prophylaxis or amelioration of cancer in a vertebrate host by administration of a hormone, drug, prodrug, toxin, cytotoxin, immunogen or DNA analogue, which process comprises the parenteral administration to said host of a therapeutically effective amount of a nanoparticle complex according to the invention.

According to a sixth aspect of the present invention there is provided the use of a nanoparticle complex of the invention in the preparation of a medicament for the treatment, prophylaxis or amelioration of disease, preferably cancer.

According to a seventh aspect of the present invention there is provided a method of delivering an active substance to a tumor or cancer cell comprising contacting said tumor or cancer cell with a nanoparticle complex comprising a nanoparticle to which is coupled a targeting molecule, said nanoparticle enclosing an active substance, and wherein said targeting molecule is folic acid, or an analogue thereof possessing binding activity for the folic acid receptor. The method of delivering the active substance may be achieved *in vivo* by administering the nanoparticle complex to a host, preferably a vertebrate host, of said tumor or cancer cell.

The nanoparticle complex of the invention contains one or more molecules of an active substance to be delivered, the nanoparticle being coupled to the TM to give a complex capable of amplified delivery of the active substance.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Brief Description of the Figure

The present invention will now be described by way of example only and with reference to the figure wherein:

Figure 1 represents the biodistribution of control and folate coated IBCA nanoparticles in tumor-bearing mice. The recovery of Folate-coated F127 IBCA nanoparticles is compared to the recovery of a control Folate-PEG-C18 complex from Balb/C mice injected with hybridoma tumor cells. The data is presented as the percentage of recovered injected counts, and shows that the quantity of nanoparticles targeted to the scTumor increased in the presence of the surface folate of the nanoparticle complexes of the invention.

Detailed Description of the Invention

The nanoparticle complexes of the present invention have been especially targeted to tumor and cancer cells using folic acid or analogues thereof as the targeting moiety. The drug is able to be released from the nanoparticle to the circulatory or lymphatic drainage system, and most preferably to the target tissue of the host. Whilst it is possible these nanoparticle complexes could be used for oral delivery of the drug to the circulatory or lymphatic drainage system in general, the products of this invention and a co-pending polymer delivery invention (Australian provisional patent application No. PQ0147 entitled "Application of Folate-mediated Targeting to Tumor Cells Using Polymers" filed on 4 May 1999 and incorporated herein in its entirety by reference) preferably relate to targeting the active substances to tumor/cancer cells.

Two basic forms of nanoparticles have been developed, nanocapsules (or microcapsules) and nanospheres (or microspheres), for enclosing, holding or containing active substances.

The term "nanoparticle" as used throughout the specification refers to a small sphere, capsule or pellet ranging in size typically from 1 nanometer to 100 micrometers in size.

The target molecules utilised in the invention are folate molecules or analogues thereof which possess binding activity for the folic acid receptor, and in particular to surface folate receptors on tumor cells. Analogues contemplated herein include, but are not limited to, modification to the ring structure, functional groups or side chains of the folic acid molecule including the additional removal of protecting groups and salts and complexes thereof derived from any source such as being chemically synthesised or identified by screening process such as natural product screening provided that the analogue possesses some binding activity for the folic acid receptor.

The active substance to be delivered is preferably a hormone, drug, prodrug, toxin, pharmaceutically active protein, immunogen, or DNA or RNA analogue.

In an embodiment of the invention there is provided a complex between folic acid and a biodegradable nanoparticle in which is trapped a toxin or cytotoxic agent as the active substance.

Suitable toxins, according to the invention, include, but are not limited to, ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, riosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin and maytansinoid.

Suitable cytotoxic agents, according to the invention, include, but are not limited to alkylating agents such as chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, 2-(hydroxymethyl)anthraquinone; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel. Other agents include DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-methyl-THF, anguidine,

maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

Other active substances which may be delivered by the folate nanoparticles of the invention include but are not limited to hormones and bioactive peptides and polypeptides, antibiotics, antipyretics, analgesics and antiinflammatory drugs, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer drugs, antidepressants, antiallergic drugs, cardiotonic drugs, antiarrythmic agents, vasodilators, antihypertensives, anticoagulants and haemostatic agents as known in the art.

In essence the nanoparticles can be formed by any number of methods, several of which are outlined below:-

(i) Solvent Evaporation

In which a compound which is soluble in one solvent is dispersed into another miscible solvent and the first solvent is evaporated off. Particles formed in this fashion have been used to administer (parenterally) a number of water insoluble compounds. An example of such a system is the formation of polyalkylcyanoacrylate nanocapsules in which the anticancer agent 5-fluorouracil is entrapped.

(ii) Desolvation

In this method a compound is dissolved in a liquid in which it is soluble (the solvent) and a second liquid (which is miscible with the first liquid, but in which the compound is not soluble) is added to the solvent. As more of the second liquid is added the compound becomes desolvated. During the process of desolvation the compound rich phase (the coacervate) contains an enriched amount of compound which is dispersed as microdroplets in the compound deficient phase. At this stage the coalesced material can be chemically crosslinked by a suitable crosslinking agent to form micro- or nano-particles, such as nanoparticles of gelatin or borine serum albumin (BSA). Solutions of these proteins are desolvated by the addition of sodium sulfate or ammonium sulfate solutions. At the point of desolvation there is an increase in turbidity, at which time the nanoparticles

can be formed by the addition of a suitable cross-linker such as glutaraldehyde or butanedione. Alternatively a biodegradable cross-linker could be employed, such as a linker containing a disulfide bond, an azo-bond or an esterase cleavable bond.

(iii) Complex coacervation

In this procedure two polyelectrolytes having opposite charge are mixed in an aqueous medium so that a spontaneous liquid/liquid phase separation occurs. The phenomenon is limited to polymers having a suitable ionic charge density and chain length. Typically these nanoparticles are formed by the addition of a polyanion such as gum arabic, alginate, or polyphosphate, to a polycation such as gelatin. Suitable particles are readily formed by the complexation of gelatin and carboxymethyl cellulose. The rate of release of pharmaceutical from such complexes can be controlled by the addition of a suitable cross-linker such as glutaraldehyde or butanedione. Alternatively a biodegradable cross-linker could be employed, such as a linker containing a disulfide bond, an azo-bond or an esterase cleavable bond.

(iv) Polymer/polymer incompatability

This procedure is based upon the observation that two chemically different polymers dissolved in a common solvent are usually incompatible. Thus the mixture will tend to form two phases. The insoluble phase can be used to coat core particles to form microcapsules. An example would be the precipitation of ethyl cellulose from cyclohexane by the addition of polyethylene.

(v) Interfacial polymerisation

In this technique, two reactants, each dissolved in a mutually immiscible liquid, diffuse to the interface between the two liquids where they react to form a capsule wall. An example of such capsule formation occurs when a mixture of sebacoyl chloride dissolved in an oil phase is emulsified into an aqueous phase containing ethylenediamine.

Polymers suitable for the formation of nanoparticles by solvent evaporation (in liquid drying) include, amongst others, poly-lactic acid, poly-(lactide/co-glycolide), poly-

hydroxybutyrate, poly-hydroxyvalerate, poly-(hydroxybutyrate/valerate), ethyl cellulose, dextran, polysaccharides, polyalkylcyanoacrylate, poly-methyl-methacrylate, poly(e-caprolactone) and various combinations and co-polymers of the above.

Polymers suitable for the formation of nanoparticles by interfacial precipitation/polymerisation include, amongst others, EUDRAGIT™; poly(N^{α},N^{ε}-L-lysinediylterephthaloyl); polymers formed by the reaction of Lysine hydrochloride and p-phthaloyl dichloride; by the reaction of acryloylated maltodextrin or acryloylated hydroxyethyl starch with ammonium peroxodisulfate and N,N,N',N'-tetramethylethylenediamine. Nanoparticles can also be formed by the polymerisation of various diamines such as ethylene diamine, phenylenediamine, toluene diamine, hexamethylene diamine, or diols such as ethylene diol, bisphenol, resorcinol, catechol, pentanediol, hexanediol, dodecanediol, 1,4-butanediol, with diacid chlorides such as sebacoylchloride and adipoyl chloride, or diisocynates such as hexamethylene diisocyanate using the methods fully described in EPA 85870002.4 and as incorporated herein by reference.

Polymers suitable for the formation of nanoparticles by polymer phase separation include co-poly(vinyl chloride:vinyl alcohol:vinyl acetate), cellulosic polymers, polyvinyl acetate, polyvinyl alcohol, polyvinylchloride, natural and synthetic rubbers, polyacrylates, polystyrene and the like. Methods to synthesize such nanoparticles are fully described in USP 4,166,800 which is incorporated herein by reference.

Polymers suitable for the formation of nanoparticles by complex coacervation include, amongst others, mixtures of polyanions, such as gum arabic, alginate, carboxymethyl cellulose, carboxymethyl starch, polystyrene sulfonic acid, polyvinyl sulfonic acid, poly-D-glucuronic acid, poly-pyruvic acid, carrageenan, heparin sulphate, polyphosphate with polycations, such as polylysine, gelatin.

Polymers suitable for the formation of nanoparticles by polymer/polymer incompatability include, amongst others, ethyl cellulose, ethylene vinyl acetate polymer, poly(lactide), or

poly(vinylidene chloride) mixed with polymers such as polyethylene, silicone, polyisobutylenes or polybutadiene.

Other materials suitable for formation of nanoparticles include, starch, cross-linked albumen, polyacrylamide, cross-linked gelatin and others obvious to those skilled in the art of nanosphere preparation.

The cross-linking agent may contain a disulfide bond or be cleavable by acid, base or periodate. Examples of suitable cross-linking agents include: N-(4-azidophenylthio)phthalimide; 4,4'-dithiobisphenylazide; dithiobis(succinimidylpropionate); dimethyl-3,3'-dithiobispropionimidate.2HCl; 3,3'-dithiobis-(sulfosuccinimidylpropionate); ethyl-4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimidate.HCl; N-succinimidyl-(4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate; sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate; N-succinimidyl-3-(2-pyridylthio)propionate; sulfosuccinimidyl-(4-azidophenyldithio)-propionate; 2-iminothiolane; disuccinimidyl tartrate; and bis-[2-(succinimidyloxycarbonyloxy)-ethyl]-sulfone.

Suitable linking of the carrier to the nanoparticles may be achieved by reaction of the carrier with a carbodiimide and *N*-hydroxysuccinimide (NHS), and then reacting the NHS derivative with a suitable functional group on the nanoparticle.

Reference to the term "folate" as used herein is to be considered in its broadest context and refers to the carboxylic acid anion of folic acid and, where not stated, the counter cation may be any suitable cation including pharmaceutically acceptable cations and may also include a proton, i.e. folic acid. The term "folate" may be taken to include reference to analogues of the folate molecule, such as methotrexate, and preferably where the analogue possesses some binding activity for the folic acid receptor.

Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a host is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition.

The subject of the treatment or prophylaxis is preferably a mammal such as but not limited to human, primate, livestock animal (e.g. sheep, cow, horse, donkey, pig) companion animal (e.g. dog, cat) laboratory test animal (e.g. mouse, rabbit, rat, guinea pig, hamster) captive wild animal (e.g. fox, deer). Preferably the mammal is a human or primate. Most preferably the mammal is a human.

It will be understood that those skilled in the art will be able to employ methods commonly known in the art for preparing suitable medicaments in concentrations and presented in forms appropriate to the administration of the folate complexes of the invention, optionally with other active agents as required, in suitable treatment regimes to achieve the desired physiological effects on the vertebrate host to be treated.

In accordance with these methods, the agents herein defined may be coadministered with one or more other compounds or molecules. For example, the nanoparticle complex of the invention may be administered in combination with folate polymer complexes, other chemotherapeutic agents or other ameliorative active substances. By "administered in combination" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the formulations. These agents may be administered in any order.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be

incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, gels, pastes, viscous colloidal dispersions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; buffering agents such as sodium bicarbonate to neutralise or buffer stomach acid; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

Administration of the agent in the form of a pharmaceutical composition may be performed by any convenient means. The agent of the pharmaceutical composition is contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. Variation depends for example, on the human or animal and the agent chosen. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The agent may be administered in any suitable manner. Routes of administration include, but are not limited to, respiratorally, intratracheally, nasopharyngeally, intravenously, intraperitoneally, subcutaneously, intracranially, intradermally, intramuscularly, intraoccularly, intrathecally, intracereberally, intranasally, infusion, orally, rectally, *via* IV drip, patch and implant. With respect to

intravenous routes, particularly suitable routes are via injection into vessels which supply the tumour or diseased organs. Peptides may also be installed into cavities for example the pleural or peritoneal cavity or injected directly into tumour tissues.

The invention is further described with reference to the following examples which are in no way limiting on the scope of the invention.

Example 1 Preparation of nanoparticles

Nanoparticles can be formed by a number of techniques common to those knowledgeable in the art, including :- solvent evaporation, complex coacervation, polymer/polymer incompatibility, gelation, interfacial polymerisation and thermal denaturation.

An effective amount of the complex is formulated with a pharmaceutically acceptable carrier, diluent or excipient to provide a medicament for administration to a patient requiring treatment of the conditions outlined in the body of the specification. The formulation is prepared using standard pharmaceutical techniques.

It is recognised that a number of factors will affect the determination of an appropriate dosage for a particular host. Such factors include the age, weight, sex, general health and concurrent disease states of the host. The determination of the appropriate dose level for the particular host is performed by standard pharmaceutical techniques.

Example 2 Preparation of nanoparticles by Coacervation.

Almost any protein can be used as the matrix for entrapping drug via the desolvation technique, however preferred proteins according to the invention include bovine serum albumen (BSA), Ovalbumen (OA) and collagen. Nanoparticles were prepared by coacervation of BSA following desolvation, according to the method of Oppenheim (Oppenheim, 1984, Oppenheim et al 1984, 1982), Briefly a 40% w/w ammonium sulphate solution was added dropwise to a solution of 1% BSA containing 0.5% w/w Tween 20 and the turbidity monitored by Klett readings, until the turbidity rose rapidly. At this point (determined by experimentation) the solution was placed in an ultra-turrax and 600 µl of

glutaraldehyde was added to cross-link the nanoparticles. Cross-linking was stopped by the addition of a solution of 12% w/w sodium metabisulfite.

Particles were then washed repeatedly with distilled water prior to coupling to the N-hydroxysuccinimide (NHS)-derivative of folic acid

Example 3 Incorporation of 5-fluorouracil

The antimitotic 5-fluorouracil was incorporated into the nanoparticle of Example 2 by dissolving 5-fluorouracil at 10 g/100 ml of the BSA/Tween solution. Desolvation and cross-linking was carried out as described in Example 2.

Example 4 Coupling of folate to nanoparticles

Proteinaceous nanoparticles (prepared by the method of Example 2) were surface coated with folate molecules by reaction of folate with 1-ethyl-3-(3-dimethylaminopropyl)carbodiomide (EDAC) and NHS followed by addition to the preformed nanoparticles.

Example 5 Preparation of folate-lipid complexes for hydrophobic insertion into nanoparticles

In order to link folate to the surface of nanoparticles which have no readily available chemical groups suitable for chemical conjugation, it is possible to prepare a complex of folate to an hydrophobic moiety which can insert, non-covalently, into the surface of the nanoparticles. Such a molecule is easily added at the time of formation of the nanoparticles. The strength of the hydrophobic association is such that there is only a very slow dissociation of the folate from the nanoparticles under physiological conditions.

a) Preparation of folate-phosphatidyl ethanolamine (folate-PEA)

Phosphatidylethanolamine (100 mg) was dissolved in 2 ml chloroform/methanol (50:50, v/v). Folate (100 mg) was added to the mixture. The folate was then cross-linked to the PEA by the addition of 200 mg of the carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC or EDAC). The reaction was allowed to proceed for 90 minutes prior to the addition of the folate-PEA to nanoparticles.

b) Preparation of other complexes between folate and an hydrophobic moiety.

Covalent complexes can be made between analogues of folate and almost any aliphatic or aromatic chains or amphipathic containing a water soluble head group suitable for conjugation and a lipid soluble tail suitable for hydrophobic association within an hydrophobic environment. Thus, any lipid (saturated, unsaturated or polyunsaturated) which has a carboxylic acid head group, such as oleic acid, octanoic acid, linoleic acid or glycerophophoric acids may be directly conjugated to an amino-folate derivative using a suitable carbodiimide (EDAC or DCC, for example). Similarly any amphiphathic molecule possessing an amino-group (amino-hexane, amino-decane, amino-dodecane, phosphatidyl-ethanolamine, may be conjugated directly to carboxy-folate using carbodiimides.

Example 6 Preparation of folate-Nanoparticles by solvent evaporation.

a) Preparation of folate-PEA-[Polymethylmethacrylate] nanoparticles

Polymethylmethacrylate (PMM, Polysciences)(MW 12,000; 500 mg) was dissolved in 2 ml of dichloromethane (DCM). The PMM in DCM was then added dropwise to 20 ml of 0.25% w/w Polyvinylalcohol (PVA) while homogenizing at 13,500 rpm with a Janke & Kunkel Ultraturrax. After 1 minute, 200 µl of folate-PEA was added and stirred gently overnight. The pink nanoparticles were then harvested by centrifugation, washed three times with water and lyophilised.

b) Preparation of folate-[PEA-Poly-lactic acid] nanoparticles.

Poly-lactic acid (PLA, Polysciences)(MW 50,000; 500 mg) was dissolved in 3 ml of DCM and then homogenised into 20 ml 1% PVA at 13,500 rpm on Ultraturrax T25 with an S25F probe for 5 minutes. Folate-PEA (400 µl) was added while the solution was stirred gently. Nanoparticles were harvested as described above.

c) Preparation of folate-PEA-[Poly-Hydroxy-butyrate/valerate] nanoparticles

Poly-hydroxy-butyrate/valerate (9% valerate) (ICI; 500 mg) was dissolved in 5 ml of DCM and homogenised into 20 ml 1% PVA at 13,500 rpm on Ultraturrax T25 with an S25F

probe for 5 minutes. Folate-PEA (400 µl) was added and the spheres processed as described in Example 8b.

Example 7 Covalent conjugation of folate to nanoparticles with surface carboxyl groups.

A general method for the conjugation of folate to the surface of nanoparticles made from polymers with free carboxyl groups is outlined below. The specific example utilises commercially available carboxyl-modified nanoparticles.

Polysciences Fluoresbrite™ carboxylate Nanoparticles (2.5% Solids Latex) were obtained from Polysciences in sizes of 0.045 µm, 0.49 µm, 2.2 µm and 9.97 µm. One ml of each of the preparations was washed extensively with distilled water and resuspended in 200 µl of distilled water. To each preparation was added 1.5 mg aminohexyl folate then 5 mg of EDAC. Each preparation was allowed to react overnight, after which unreacted material was removed by repeated washing with distilled water or by dialysis against distilled water.

Example 8 Surface derivatization of nanoparticle

Many polymers used in the preparation of nanoparticles by solvent evaporation do not contain functional groups for direct conjugation to folate or its functionalised analogues, however it is possible to modify the surface of the preformed nanoparticles to introduce functional groups suitable for conjugation to folate.

a) Surface derivatization of polylactic acid (PLA) nanoparticles

Preformed PLA nanoparticles (10 mg) were gently suspended in distilled water (350 µl) by rotation on a rotary shaker for 2 hours. Hydrazine hydrate (10 µl) was added and the suspension was shaken overnight at room temperature. The spheres were spun down and repeatedly washed with water by re-suspension and centrifugation. The washing procedure was repeated until the supernatant failed to give a positive hydrazine test (purple colour upon reaction with a solution of trinitrobenzenl sulphonate (TNBS); 1 mg/ml). The spheres were washed a further two times and the wet pellet used directly for conjugation to folate.

b) Conjugation of folate to hydrazine modified PLA nanoparticles

A sample of the hydrazine modified PLA nanoparticles (3 µl wet pellet) was suspended in distilled water (250 µl). Aqueous solutions of folate (10 mg/ml, 400 µl) and EDAC (100 mg/ml, 100 µl) were added and the reaction mixture shaken overnight at room temperature. The suspension was spun down and the supernatant removed. The pellet was washed repeatedly with distilled water (6 washes). The residual pellet, which was pale pink in colour, was vacuum dried.

Comparative Examples

Two control reactions were performed concurrently with the above conjugation. In the first a 3 mg sample of hydrazine-modified PLA nanoparticles was treated with the folate as described above but distilled water was used in place of the EDAC solution. In the second control a 2 mg sample of unmodified PLA nanoparticles was treated with both folate and EDAC as described above. For both controls the pellet remaining after repeated washing was a clear white colour with no evidence of any associated folate.

Example 9 Preparation of isobutyl-cyanoacrylate (IBCA) nanocapsules, surface-coated with folate

Nanocapsules suitable for biodistribution studies were prepared with ^{125}I-insulin as ant internal marker. Briefly, 10 mg insulin was dissolved at 10mg/ml in 0.1M HCl. An aliquot (1µl) of ^{125}I-insulin was added to the cold insulin, which was mixed with 100µl miglyol and vortexed. EtOH (10 ml) was added to the insulin/miglyol mix and mixed by vortexing. Isobutyl-cyanoacrylate IBCA (100 µl, Sicomet) was added to the clear solution, which was immediately added to 60 ml 0.25% F-127. After 30 minutes the preparation was split into 2 equal halves. One half was left to stir overnight, whilst to the other half was added 27mg folate-PEG-octadecanoic acid (80 mg/ml in EtOH). The solution was left to stir overnight. Both solutions were then treated in a similar fashion. Large aggregates were removed by centrifugation at 10K rpm for 20 minutes. Both particle preparations were concentrated and washed in a Amicon positive pressure filtration unit using a 300,000 MW cut off membrane. Particles were stabilised by surface cross-linking with di-succinimidyl-2-aminoethyl-2-amino-2-benzyl-ethanoate (DSAB). DSAB was converted to the NHS-ester as follows. DSAB (40 mg) was dissolved in an equal weight of DMF, to

which was added NHS (24 mg, 240µl DMF). DCC (Dicyclohexylcarbodiimide, 44mg, 440µl, made up fresh) was then added to the DSAB mixture and allowed to activate for 20 min while stirring rapidly. The DSAB-NHS-ester was added at 0.32 mg per 2.1 mg nanocapsules, and left to stir overnight. The particles were then dialysed before use in biodistribution studies.

Example 10 Demonstration of folate-mediated targeting of nanoparticles.

In order to examine the potential utility of folate as a targeting agent for nanoparticles, folate derivatized IBCA nanoparticles were prepared as described in Example 9. Control nanoparticles were prepared without folate. For biodistribution studies, Balb/C mice were injected subcutaneously with 2×10^6 hybridoma tumour cells. Two weeks after tumour injection, the radio-labelled nanoparticles were injected intravenously into the mice. At various time-points the mice were bled from the retro-orbital plexus, euthanased and their tissues removed for determination of radioactivity. Data is presented in Figure 1 as the percentage of injected counts that were injected in the mice.

A small but lower quantity of uptake of unmodified nanoparticles into the tumour was found as can be seen from Figure 1, the quantity of nanoparticles targeted to the tumour increased in the presence of surface folate.

Industrial applications

The present invention provides a simple and novel technique for the amplification of the folate uptake system, thus enabling the amplified delivery of a wide range of active agents to tumor and cancer cells in particular.

References

Oppenheim R.C. (1984) in "Polymeric Microparticles" (Guiot, P and Couvreur, P. Eds.) CRC Press, Boca Raton.

Oppenheim R.C., Gipps, E.M. Forbes, J.F. and Whitehead R.H. (1984) in "Nanoparticles and Drug Therapy" (Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. Eds) Elsevier Science Publishers B.V.

Oppenheim, R.C., Stewart, N.F., Gordon, L. and Patel, H.M. (1982) Drug Devel. Indust. Pharm. 8: 531-546.

Allen, R.H. and Majerus, P.W. (1972) J.Biol. Chem. 247: 7702-7717.

Yamada, R.-H and H.P.C. Hogenkamp. (1972) J.Biol.Chem. 247 : 6266-6270.

The claims defining the invention are as follows:

1. A complex comprising a nanosphere or nanocapsule, to which is coupled a targeting molecule, said nanosphere or nanocapsule enclosing an active substance, and wherein said targeting molecule is folic acid, or an analogue thereof possessing binding activity to the folic acid receptor.

2. A complex of claim 1 wherein the active substance is a hormone, drug, prodrug, pharmaceutically active protein, immunogen, or DNA or RNA analogue.

3. A complex of claim 2 wherein the active substance is a toxin, cytotoxic agent or prodrug thereof.

4. A complex of claim 3 wherein the toxin is selected from the group consisting of ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, and ribosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin, maytansinoid.

5. A complex of claim 3 wherein the cytotoxic agent is selected from the group consisting of chlorambucil, cyclophosphamide, melphalan, cyclopropane, doxorubicin, daunomycin, adriamycin, mitomycin C, [2-(hydroxymethyl)anthraquinone], methotrexate, dichloromethatrexate: cisplatin, carboplatin, metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel, DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

6. A complex of claim 1 wherein the nanosphere or nanoparticle is prepared by solvent evaporation, complex coacervation, polymer/polymer incompatibility, gelation, interfacial polymerisation or thermal denaturation.

7. A complex of claim 6 wherein the nanosphere or nanoparticle is biodegradable.

8. A process for the production of a complex of claim 1, which process comprises one or more of the following steps:

   a. reacting nanoparticles with a targeting molecule to form the complex;
   b. chemically modifying a targeting molecule to provide at least one functional group capable of forming a chemical linkage and reacting nanoparticles and the modified targeting molecules to form the complex;
   c. reacting nanoparticles with at least one cross-linking agent to prepare "activated" nanoparticles which are reacted with a targeting molecule to form the complex;
   d. reacting a targeting molecule with at least one cross-linking agent and reacting the nanoparticles with the reacted targeting molecule to form the complex;
   e. reacting nanoparticles and a targeting molecule with at least one cross-linking agent to the complex;
   f. reacting nanoparticles with at least one cross-linking agent, reacting a targeting molecule with at least one cross-linking agent and reacting the reacted nanoparticles and the reacted targeting molecule to form the complex; or
   g. reacting a targeting molecule with at least one cross-linking agent to prepare an analogue which is reacted with a hydrophobic moiety to form a hydrophobic derivative of the targeting molecule, and then incubating the hydrophobic derivative of the targeting molecule with a nanosphere in such a manner that the nanosphere is coated hydrophobically with the targeting molecule.

9. A process of claim 8 wherein the cross-linking agent contains a disulfide bond or is cleavable by acid, base or periodate.

10. A process of claim 8 wherein the cross-linking agent is selected from the group consisting of N-(4-azidophenylthio)phthalimide, 4,4'-dithiobisphenylazide, dithiobis(succinimidylpropionate), dimethyl-3,3'-dithiobispropionimidate.2HCl, 3,3'-dithiobis-(sulfosuccinimidylpropionate), ethyl-4-azidophenyl)-1,3'dithiopropionate, sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimidate.HCl, N-succinimidyl-(4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate, N-succinimidyl-3-(2-pyridylthio)propionate, sulfosuccinimidyl-(4-azidophenyldithio)-propionate, 2-iminothiolane, disuccinimidyl tartrate and bis-[2-(succinimidyloxycarbonyloxy)-ethyl]-sulfone.

11. A process of claim 8 wherein the targeting molecule is cross-linked to the nanosphere or nanoparticle by reaction of the carrier with a carbodiimide and *N*-hydroxysuccinimide (NHS), and then reacting the NHS derivative with a suitable functional group on the nanosphere.

12. A process of claim 8 wherein the cross-linking agent contains a biodegradable bond

13. A process of claim 12 wherein the cross-linking agent is cleaved by an esterase, glutathione, or azo-reductase.

14. A complex prepared by a process of claim 8.

15. A pharmaceutical composition comprising a complex of claim 1 or claim 14 in association with a pharmaceutically acceptable carrier, excipient, diluent, or adjuvant.

16. A method for the treatment or prophylaxis of disease in a vertebrate host, including a human, which method comprises the administration to said host a therapeutically

effective amount of a complex of claim 1 or 14 or a pharmaceutical composition of claim 15.

17. A method of claim 16 wherein the disease is cancer.

18. A method of claim 16 wherein the administration is parenteral administration.

19. A method of claim 16 wherein the administration is oral administration.

20. Use of a nanoparticle complex of claim 1 or claim 14 in the preparation of a medicament for the treatment, prophylaxis or amelioration of disease.

21. Use of claim 20 wherein the disease is cancer.

22. A method of delivering an active substance to a tumor or cancer cell comprising contacting said tumor or cancer cell with a nanoparticle complex comprising a nanoparticle to which is coupled a targeting molecule, said nanoparticle enclosing the active substance, and wherein said targeting molecule is folic acid, or an analogue thereof possessing binding activity for the folic acid receptor.

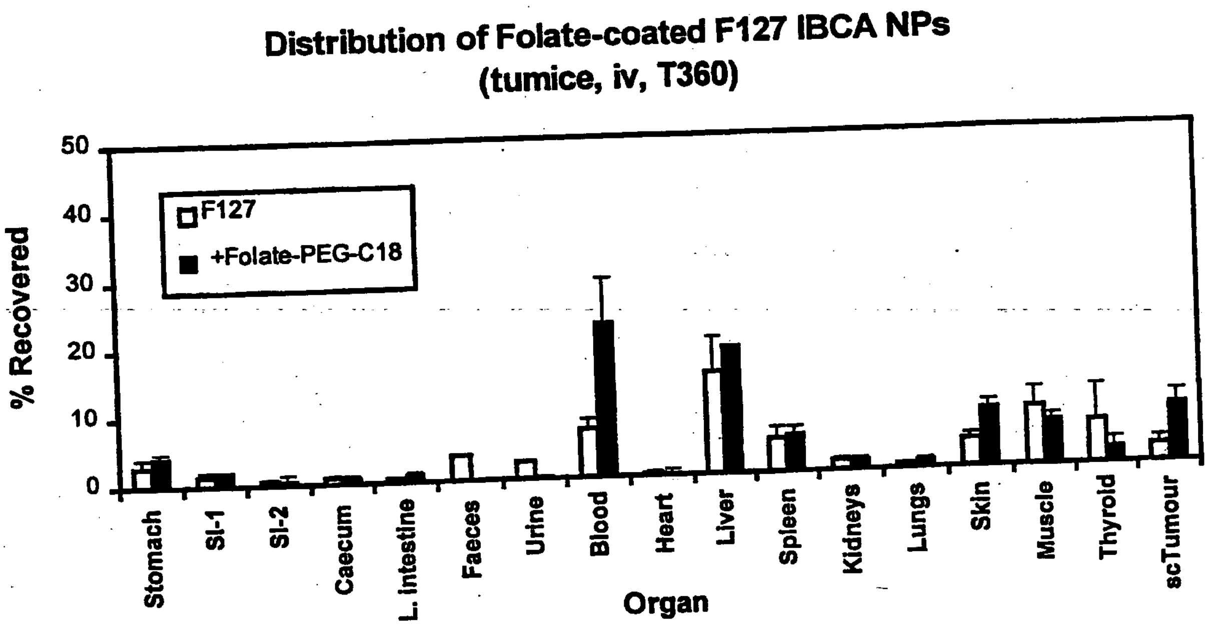
Distribution of Folate-coated F127 IBCA NPs
(tumice, iv, T360)
50
40
30
20
10
0
% Recovered
F127
+Folate-PEG-C18
Stomach
SI-1
SI-2
Caecum
L. Intestine
Faeces
Urine
Blood
Heart
Liver
Spleen
Kidneys
Lungs
Skin
Muscle
Thyroid
scTumour
Organ

Figure 1

1/1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00405

A. CLASSIFICATION OF SUBJECT MATTER	

Int. Cl. 7: A61K 009/51, 047/48, 031/519; A61P 35/00; C08G 69/36, CO8B 37/02, 37/08, 11/08; C08F 120/56; C08G 71/04, 65/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
FILE WPAT AND CHEM ABS. KEYWORDS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
FILE MEDLINE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT, FILE MEDLINE AND CHEM ABS. KEYWORDS: Folate, folic, FR (w) alkha, FR (w) beta, methotrexate, drug polymer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/10038 A (APOLLON, INC.) 4 April 1996 See whole document	1-22
X	WO 97/25067 A (The University of Nottingham) 17 July 1997 See whole document	1-20
X	Anticancer Research, Vol. 17, 1997, pages 29-36 (Ginobbi P. et al.) "Folic Acid-Ploylysine carrier Improves Efficacy of c-myc Anti sense Oligodeonynucleotides on Human Melanoma (M14) cells" See whole document	1-22

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search 5 July 2000	Date of mailing of the international search report 12TH JULY 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer Arati Sardana ARATI SARDANA Telephone No : (02) 6283 2627

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Investigative Radiology, Vol. 32, No 12, December 1997, page 748-54 (Weiner E. C. et al) "Targeting Dendrimer-Chelates to Tumors and Tumor cells Expressing the High-Affinity Folate Receptor" See whole document	1-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00405

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9610038	AU	37317/95	CA	2201396	EP	789708
		US	5837533				
WO	9725067	AU	13866/97	CA	2241830	EP	871491
		GB	2324534	NO	982894		

END OF ANNEX